Journal of Stress Physiology & Biochemistry, Vol. 21, No. 2, 2025, pp. 187-194 ISSN 1997-0838

ORIGINAL ARTICLE

Biochemical Response of *Glycine max* (L.) Merr. to Lead Stress

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Received November 26, 2024

This pot experiment investigated the effects of varying concentrations of lead acetate in soil on selected biochemical parameters in soybean (*Glycine max* (L.)), Lead concentrations were set at 0 (control), 200, 400, 600, 800, and 1000 mg/kg of soil, with observations recorded at pre-flowering (30 days), peak-flowering (45 days), and post-flowering (60 days) stages. Results indicated a clear dose-response relationship between lead acetate concentration and soybean biochemical attributes. The highest levels of chlorophyll-a (0.6287 mg), chlorophyll-b (1.0193 mg), total chlorophyll (1.6480 mg), carbohydrate (53.04 mg), lipid (43.23 mg), and protein content (9.00 mg) were recorded at 200 mg/kg during the post-flowering stage. In contrast, increasing lead concentrations led to significant reductions in all biochemical parameters compared to the control group, with the most severe impacts observed at 1000 mg/kg where chlorophyll-a (0.2961 mg), chlorophyll-b (0.4889 mg), total chlorophyll (0.7850 mg), carbohydrate (27.08 mg), lipid (33.92 mg), and protein content (3.897 mg) significantly declined. These findings underscore the toxic impact of lead acetate on soybean growth, highlighting the urgent need for effective mitigation strategies to address lead contamination, thereby promoting agricultural sustainability and protecting public health.

Key words: Heavy metal, lead (Pb), Chlorophyll, Carbohydrate, Lipid, Protein, pre-flowering stage, peak-flowering stage, post-flowering stage

The rising levels of lead contamination in agricultural soils pose a significant threat to food safety and ecosystem integrity. Lead, a toxic heavy metal, is not essential for plant growth, and its accumulation in soil can have detrimental effects on crop health. Sources of lead contamination are varied, including industrial emissions, improper waste disposal, and the application of contaminated fertilizers and pesticides (Gupta *et al.,* 2016). Once present in the soil, lead can become available to plants through complex interactions influenced by soil physicochemical properties, pH, organic matter, and microbial activity. Lead primarily enters plants through their roots, exerting inhibitory effects on crucial processes such as plant growth, water status, mineral nutrition, and enzymatic activities (Gupta and Meena, 2024). In major crops like soybean (*Glycine max* (L.)), which is vital for global food security as a primary source of vegetable oil and protein, excessive lead absorption can compromise both yield and nutritional quality. While soybean plants exhibit resilience to environmental stresses, they are particularly sensitive to heavy metal imbalances, leading to reduced growth, impaired reproductive development, and decreased seed viability (Gupta and Meena, 2024). As global populations continue to grow, maintaining the health and productivity of staple crops like soybean becomes increasingly critical. This study aims to investigate the impact of lead acetate on the biochemical parameters of *Glycine max* (L.). By examining the relationship between lead concentrations in soil and its effects on key physiological traits, the research seeks to illuminate the broader implications of lead contamination on crop quality and safety.

MATERIALS AND METHODS

Chemical

In the present investigation lead acetate $(CH3COO)_2$ $Pb.3H_2O$) is used for the treatment purpose with various concentrations ranging from 0 (control), 20, 400, 600, 800, 1000 mg/kg of soil.

Plant material

Certified seeds of soybean (*Glycine max* (L.) Merr.) variety JS-95-60 were procured from Agriculture Station, Kota, Rajasthan

Pot Experiment

The experiment was conducted in April at the University of Rajasthan's Botany Department greenhouse. Pots, 30 cm tall and 25 cm in diameter, filled with 4 kg of garden soil, were randomly placed to mitigate environmental variations. Lead acetate was applied at concentrations of 200, 400, 600, 800, and 1000 mg/kg of soil, with untreated pots as controls. Soybean seeds, sterilized with 0.1% $HgCl_2$ and rinsed with distilled water, were sown at 2 cm depth in each pot. Consistent plant numbers were maintained with alternate-day watering. Each treatment was replicated thrice across pre-flowering (30 days), peak-flowering (45 days), and post-flowering (60 days) stages to ensure robust data collection on biochemical parameters.

Biochemical analysis

Chlorophyll quantification

Chlorophyll was extracted and quantified according to Arnon's method (1949). Fresh leaves (1 g) from each treatment were macerated in 80% acetone and centrifuged at 2000 rpm for 10 minutes. The supernatant was diluted to 100 ml with 80% acetone in a volumetric flask. The amount of chlorophyll ‘a', and ‘b' were quantified by measuring the optical density at 663 nm and 645 nm wavelengths using a UV-VIS spectrophotometer. Pigment contents were calculated as mg/g FW (fresh weight).

Carbohydrate estimation

The standard Anthrone method (Yemm and Willis, 1954) was employed for carbohydrate estimation. Samples (0.1g) were hydrolyzed with 5ml 2.5N HCl in boiling water for 3 hours, neutralized with solid sodium carbonate, and centrifuged. Supernatants were collected, and aliquots (0.5ml and 1.0ml) were made up to 1ml with distilled water. Anthrone reagent (4ml) was added, and tubes were heated for 8 minutes, then cooled. Absorbance at 630nm was measured to calculate carbohydrate content (mg/g FW) using a glucose standard curve**.**

Lipid determination

Lipid content was determined using the method described by Jayaram in 1981. One gram of dried

sample was macerated with 10 ml distilled water and transferred to a conical flask with 30 ml chloroform: methanol (2:1, v/v). After overnight extraction at room temperature in the dark, 20 ml chloroform was added, followed by centrifugation. The clear lower chloroform layer, containing lipids, was collected in pre-weighed glass vials. After solvent evaporation, samples were weighed. lipids were calculated as total lipid/gm of dried sample.

Protein estimation

Protein was quantified according to Lowery (1951) method. Samples were homogenized in 0.1M phosphate buffer (pH=6.8). After adding Folin-Ciocalteau reagent absorbance was recorded at 660 nm and protein was calculated using a Bovine Serum Albumin (BSA) standard curve.

Statistical analysis

Statistical analysis employed SPSS ver. 25.0 and Microsoft Office Excel 2016. All parameters studied were expressed as mean ± standard error (S.E.) The data was analyzed by analysis of variance (ANOVA) to determine the statistical significance of the differences between means of treatments.

RESULTS

Chlorophyll-a

At pre-flowering stage, chlorophyll-a was 0.8290mg under control treatment. With increased lead concentration, it was significantly declined in comparison to a control level. It was 0.6981mg, 0.6680mg, 0.5990mg, 0.5127mg and 0.4988mg in level 200, 400, 600, 800,1000mg/kg respectively. Minimum chlorophyll-a was observed at 1000 mg/kg lead treatment, a 40 % decrease compared to control.

At the peak-flowering stage, chlorophyll-a was 0.8445mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to control. It was 0.7443mg, 0.7104mg, 0.6555mg, 0.6063mg and 0.5390mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum chlorophyll-a was observed at 1000 mg/kg lead treatment, a 36 % decrease compared to control.

At the post-flowering stage, chlorophyll-a was 0.8027mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to a control level. It was 0.6287mg, 0.5647mg, 0.3783mg, 0.3774mg and 0.2961mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum chlorophyll-a was observed at 1000 mg/kg lead treatment, a 63 % decrease compared to control (Table 1).

Chlorophyll-b

At pre-flowering stage, chlorophyll-b was 1.0615mg under control treatment. With increased lead concentration, chlorophyll-b was significantly declined in comparison to a controlled level. It was 1.0901mg, 1.0047mg, 0.9820mg, 0.9722mg and 0.6173mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum chlorophyll-b was observed at 1000 mg/kg lead treatment, a 42 % decrease compared to control

At the peak-flowering stage, the chlorophyll-b was 1.0953mg under the control treatment. With increased lead concentration, chlorophyll-b was significantly declined in comparison to a control condition. It was 1.0916mg, 1.0561mg, 1.0369mg, 0.9508mg and 0.8617mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum chlorophyll-b was observed at 1000 mg/kg lead treatment, a 21 % decrease compared to control.

At the post-flowering stage, chlorophyll-b was 1.0247mg under the control treatment. With increased lead concentration, chlorophyll-b was significantly declined in comparison to a controlled level. It was 1.0193mg, 0.8917mg, 0.7681mg, 0.6342mg and 0.4889mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum chlorophyll-b was observed at 1000 mg/kg lead treatment, a 52 % decrease compared to control (Table 1).

Total Chlorophyll (a+b)

At pre-flowering stage, total chlorophyll was 1.8905mg under control treatment. With increased lead concentration, total chlorophyll was significantly declined in comparison to a controlled level. It was 1.7882mg, 1.6727mg, 1.5810mg, 1.4849mg and 1.1161mg in level 200, 400, 600, 800, 1000mg/kg respectively. Total chlorophyll was observed at 1000 mg/kg lead treatment, a 52 % decrease compared to control.

At the peak-flowering stage, total chlorophyll was

1.9398mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to a control level. It was 1.8359mg, 1.7665mg, 1.6924mg, 1.5571mg and 1.4007mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Total chlorophyll was observed at 1000 mg/kg lead treatment, a 28 % decrease compared to control.

At the post-flowering stage, total chlorophyll was 1.8301mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to a control level. It was 1.6480mg, 1.4564mg, 1.1464mg, 1.0116mg and 0.7850mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Total chlorophyll was observed at 1000 mg/kg lead treatment, a 57 % decrease compared to control (Table 1).

Carbohydrate

At pre-flowering stage, carbohydrate was 41.56 mg under control treatment. With increased lead concentration, carbohydrate was significantly declined in comparison to a control level. It was 37.71mg, 33.65mg, 30.02mg, 27.12mg and 23.10mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum carbohydrate was observed at 1000 mg/kg lead treatment, a 44% decrease compared to control.

At the peak-flowering stage, carbohydrate was 60.22mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to a control level. It was 56.94mg, 51.26mg, 47.09mg, 41.87mg and 31.28mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum carbohydrate was observed at 1000 mg/kg lead treatment, a 48% decrease compared to control.

At the post-flowering stage, carbohydrate was 57.04mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to a control level. It was 53.04mg, 48.67mg, 41.12mg, 34.54mg and 27.08mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum carbohydrate was observed at 1000 mg/kg lead treatment, a 52% decrease compared to control (Table 2).

Lipid

At pre-flowering stage, lipid was 45.30 mg under control treatment. With increased lead concentration, lipid was significantly declined in comparison to a control level. It was 43.87mg, 41.30mg, 37.65mg, 35.72mg and 33.43mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum lipid was observed at 1000 mg/kg lead treatment, a 26% decrease compared to control.

At the peak-flowering stage, lipid was 46.48mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to a control level. It was 45.61mg, 43.52mg, 41.70mg, 39.24mg and 35.16mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum lipid was observed at 1000 mg/kg lead treatment, a 24% decrease compared to control.

At the post-flowering stage, lipid was 44.67mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to a control level. It was 43.23mg, 41.89mg, 39.55mg, 37.87mg and 33.92mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum lipid was observed at 1000 mg/kg lead treatment, a 24% decrease compared to control (Table 3).

Protein

At pre-flowering stage, Protein was 9.6512 mg under control treatment. With increased lead concentration, protein was significantly declined in comparison to a controlled level. It was 6.761mg, 6.032mg, 5.173mg, 2.934mg and 2.569mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum Protein was observed at 1000 mg/kg lead treatment, a 70% decrease compared to control.

At the peak-flowering stage, Protein was 12.332mg under the control treatment. With increased lead concentration, protein was significantly declined in comparison to a control level. It was 10.041mg, 8.713mg, 7.360mg, 5.225mg and 4.766mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum Protein was observed at 1000 mg/kg lead treatment, a 61% decrease compared to control.

At the post-flowering stage, Protein was 10.509mg under the control treatment. With increased lead concentration, it was significantly declined in comparison to a controlled level. It was 9.00mg, 7.151mg, 4.860mg, 4.600mg and 3.897mg in level 200, 400, 600, 800, 1000 mg/kg respectively. Minimum Protein was observed at

1000 mg/kg lead treatment, a 63% decrease compared to control (Table 4).

Table 1: Impact of lead on Chlorophyll (mg/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage			Peak-flowering stage			Post flowering stage		
	Chl-a	Chl-b	Total Chl	Chl-a	Chl-b	Total Chl	Chl-a	Chl-b	Total Chl
Control	0.8290± 0.012	1.0615 ±0.026	1.8905 ±0.043	0.8445± 0.034	1.0953 ±0.036	1.9398± 0.023	0.8027 ±0.023	1.0274 ±0.032	1.8301± 0.023
200 mg/kg	0.6981± 0.023 [b]	1.0901 ±0.023 [a]	1.7882 ±0.023 [b]	0.7443± 0.037 [a]	1.0916 ±0.034 [a]	1.8359± 0.023 [b]	0.6287 ±0.016 [a]	1.0193 ±0.023 [b]	1.6480± 0.034 [a]
400 mg/kg	0.6680± 0.017 [c]	1.0047 ±0.032 [c]	1.6727 ±0.032 [b]	0.7104± 0.026 [b]	1.0561 ±0.023 [c]	1.7665± 0.032 [c]	0.5647 ±0.020 [c]	0.8917 ±0.045 [b]	1.4564± 0.023 [c]
600 mg/kg	0.5990± 0.045 [c]	0.9820 ±0.045 [b]	1.5810 ±0.031 [c]	0.6555± 0.032 [c]	1.0369 ±0.021 [b]	1.6924± 0.026 [b]	0.3783 ±0.034 [c]	0.7681 ±0.056 [b]	1.1464± 0.026 [b]
800 mg/kg	0.5127± 0.49 [b]	0.9722 ±0.054 [c]	1.4849 ±0.034 [c]	0.6063± 0.043 [c]	0.9508 ±0.017 [c]	1.5571± 0.043 [c]	0.3774 ±0.032 [a]	0.6342 ±0.043 [c]	1.0116± 0.037 [c]
1000 mg/kg	0.4988± 0.039 [c]	0.6173 ±0.067 [c]	1.1161 ±0.054 [c]	0.5390± 0.023 [b]	0.8617 ±0.031 [c]	1.4007± 0.028 [c]	0.2961 ±0.029 [c]	0.4889 ±0.024 [c]	0.7850± 0.059 [c]

Values were expressed as mean± SEM, Significance level: [a]p ≤ 0.1, [b]p ≤ 0.05, [c]p ≤ 0.01

Table 2: Impact of lead on Carbohydrate (mg/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post flowering stage
Control	41.56±0.46	60.22±0.38	57.04±0.51
200 mg/kg	37.71±0.51 [b]	56.94±0.45 [c]	53.04±0.51 [c]
400 mg/kg	33.65±0.43 [c]	51.26±0.51 [b]	48.67±0.43 [c]
600 mg/kg	30.02±0.44 [c]	47.09±0.36 [c]	41.12±0.39 [c]
800 mg/kg	27.12±0.53 [c]	41.87±0.51 [c]	34.54±0.41 [c]
1000 mg/kg	23.1±0.36 [c]	31.28±0.57 [c]	27.08±0.49 [c]

Values were expressed as mean± SEM, Significance level: [a]p ≤ 0.1, [b]p ≤ 0.05, [c]p ≤ 0.01

Table 3: Impact of lead on lipid (mg/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post flowering stage
Control	45.30±0.57	46.48±0.48	44.67±0.41
200 mg/kg	43.87±0.48 [a]	45.61±0.45 [a]	43.23±0.42 [a]
400 mg/kg	41.30±0.58 [b]	43.52±0.39 [b]	41.89±0.44 [b]
600 mg/kg	37.65±0.48 [b]	41.70±0.58 [c]	39.55±0.54 [c]
800 mg/kg	35.72±0.58 [b]	39.24±0.51 [c]	37.87±0.56 [c]
1000 mg/kg	33.43±0.37 [c]	35.16±0.45 [c]	33.92±0.90 [c]

Values were expressed as mean± SEM, Significance level: [a]p ≤ 0.1, [b]p ≤ 0.05, [c]p ≤ 0.01

Table 4: Impact of lead on Protein (mg/gm) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post flowering stage
Control	9.651±0.37	12.332±0.42	10.509±0.44
200 mg/kg	6.761±0.38 [c]	10.041±0.48 [a]	9.00±0.48 [a]
400 mg/kg	6.032±0.44 [b]	8.713±0.44 [b]	7.151±0.35 [c]
600 mg/kg	5.173±0.57 [c]	7.360±0.42 [c]	4.860±0.47 [c]
800 mg/kg	2.934±0.42 [c]	5.225±0.35 [c]	4.600±0.47 [c]
1000 mg/kg	2.569±0.16 [c]	4.766±0.58 [c]	3.897±0.41 [c]

Values were expressed as mean± SEM, Significance level: [a]p ≤ 0.1, [b]p ≤ 0.05, [c]p ≤ 0.01

DISCUSSION

The current study demonstrates that with increasing concentrations of lead biochemical parameters such as chlorophyll, carbohydrate, lipid and protein are decreased during the pre, peak, and post-flowering stages while application of 1000 mg/kg resulted in highest reductions in parameters across all growth stages compared to the control. Photosynthesis, an important process for plant growth and biomass production, was used as an indicator of early stress. The photosynthetic system is very sensitive to the toxicity of heavy metals. This has been ascribed to the metal disturbing action on chlorophyll synthesis, activity of photochemical enzymes and plant water balance (Maxwell and Johnson, 2000). The result of the present study is indicated that lead caused certain degrees of negative effects on chlorophyll synthesis with increasing concentration. Its deleterious effect became more pronounced by the higher concentrations. This decline in chlorophyll content might be caused as substitution of the central Mg in chlorophyll by heavy metals. It causes inhibition of important enzymes associated with chlorophyll biosynthesis, such as *δ*- aminolaevulinic acid dehydrogenase (ALAD) and protochlorophyllide reductase (Shankers *et al*., 2005). There have also been many researches which were conducted on the effects of lead on chlorophyll synthesis (Wei *et al.,* 2009), showing that lead stress with certain concentrations exerted inhibitory effects on chlorophyll content in plant leaves. In fact, lead toxicity is reported to decrease in chlorophyll content in several plant species, *Phaseolus vulgaris* cv Strike (Fikriye, 2006), *Vigna radiata* (Gautam *et al*., 2008), *Brassica juncea* (John *et al.,* 2009), *Lemna minor* (Jayasri *et al.,* 2017). Proteins are the beginners and builders of biochemical reactions. These are the integral part of protoplasm and vary in their content from plant to plant which is dependent on the growth and differentiation of plants (Thomsen *et al*., 1991).in the present study, It was observed that the protein content gets reduced in the test plants when exposed to increasing concentration of Pb stress. Our results agree with the findings of other workers (Balestrasse *et al*., 2003; Bavi *et al*., 2011) in different crop plants. The reduction in the amount of protein could be due to decrease in protein synthesis or an increase in protein degradation (Balestrasse *et al*., 2003). The decrease in protein content in *L. polyrrhiza* may be caused by enhanced protein degradation process as a result of increased protease activity (Palma *et al*., 2002) that is found to increase under stress conditions. Jayasri *et al.,* (2017) reported that lead toxicity significantly reduced the synthesis of protein in *Lemna minor* plants. Earlier reports also revealed that lead stress decreases the protein concentration in *Brassica napus* (Gohari, 2012), *Triticum aestivum* cv. Maruca (Zenovia *et al*., 2011), *Brassica juncea* (John *et al.,* 2009). *Vigna radiata* (Gautam *et al*., 2008). Carbohydrates are the polyhydroxy aldehydes or polyhydroxy ketones which are widely distributed in plants as changes in the carbohydrate fractions during growth and development are consistent with the changes in photosynthetic pigments. In present study Pb toxicity caused a marked reduction in carbohydrate content of soybean. These results are similar with the findings of several other workers. Considerable reduction in the carbohydrate content of *Lemna polyrrhiza* (L.) was observed when treated with different concentrations of lead (John *et al*., 2009). Similar results were also reported by (Gaweda, 2007) in vegetables and Hamid *et al*. (2010) in *Phaseolus vulgaris* when treated with different concentrations of lead. The massive decrease in carbohydrate in lead treated plants may be due to the inhibition of chlorophyll biosynthesis leading to a decrease in carbohydrate contents. Lipids are the supporters and storage molecules of cells. These are greasy materials which play important cellular structures. Biological membranes are the structures that are mostly formed by lipid and protein. In present study, under different treatments of lead in the study, lipid content was decreased with increasing concentrations of lead as compared to the control. It was also reported by Manivasagaperumal *et al.* (2011) in heavy metal treated Cluster Bean (*Cyamopsis tetragonoloba* (L.) Taub). These consistent findings underscore the reliability of the observed trends and highlights the importance of implementing careful management practices to mitigate

the adverse effects of lead exposure in agricultural environments.

CONCLUSION

The study findings indicate that lead imposition significantly influenced various biochemical parameters throughout different growth stages of *Glycine max* (L.) Merr. (soybean). Lowest concentrations of lead i.e. 200 mg/kg was not showing significant reducing effect on pigment composition, carbohydrate, lipid, and protein content. However, as lead concentration increased, these parameters showed a gradual decline, with the most pronounced reductions observed at 1000 mg/kg. Lipid content was relatively less affected compared to other parameters. Chlorophyll, carbohydrate, and protein levels decreased under higher lead exposure. The study recommends avoiding lead concentrations exceeding 400 mg/kg in soil to mitigate potential phytotoxicity and nutrient imbalances in soybean. The highest concentration tested (1000 mg/kg) had the most adverse effects across all parameters studied. It underscores the importance of informing farmers about soil heavy metal levels. For future research, strategies focusing on enhancing enzymes that remove reactive oxygen species (ROS) and increasing antioxidant compounds are proposed to improve oxidative stress tolerance in plants exposed to Lead pollution on agricultural lands. These approaches could potentially mitigate the detrimental effects observed in this study.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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